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Characteristics of defaunated soil

I. A comparison of three techniques applied to two different forest soils

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With 5 figures

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1. Introduction

The effects of soil fauna on respiration, decomposition, and nutrient turnover have been a subject of considerable interest in recent years (CROSSLEY, 1977; PETERSEN & LUXTON, 1982, COLEMAN *et al.*, 1983; SEASTEDT, 1984; ANDERSON *et al.*, 1985; INGHAM *et al.*, 1985). In addition, there has been increasing interest in population interactions and community or food web dynamics in soil ecosystems (ANDERSON, 1978, SANTOS *et al.*, 1981, HENDRIX *et al.*, 1986; MOORE *et al.*, 1988). Experimental studies of the effects of fauna in soil ecosystems require that the faunal populations of interest be manipulated in some way; either increased, reduced, or eliminated completely. Techniques involving physical exclusion (small-mesh bags) or toxic chemicals have commonly been employed to manipulate soil fauna, especially in the field (reviewed by SEASTEDT, 1984; see also INGHAM *et al.*, 1985; INGHAM *et al.*, 1986; WRIGHT & COLEMAN, 1988). Microcosm experiments provide the opportunity for complete control over the species and interactions examined ("gnotobiotic" systems; ELLIOTT *et al.*, 1979; HANLON & ANDERSON, 1979; INGHAM *et al.*, 1985; ALLEN-MORLEY & COLEMAN, 1989).

As a technique for studying soil ecosystems, defaunation offers the following advantages:

(1) If defaunation is complete and reinvasion is prevented, faunal populations cannot recover, and therefore the treatment can in principle be maintained indefinitely.
(2) Selected taxonomic or trophic groups can be reinoculated singly or in combinations to test particular hypotheses.

The major disadvantages of defaunation are technical ones: defaunation techniques may be difficult or expensive to apply, or cause undesired changes in soil physical or chemical characteristics.

Here we present an evaluation of three convenient defaunation techniques: microwaving, deep freezing in combination with drying, and biocide application. Microwaving has previously been used against a wide variety of organisms in soil; it acts primarily through rapid heating (FERRISS, 1984; OU *et al.*, 1985). Both microwaving and deep-freezing and drying are more effective if repeated at intervals, since this allows resistant forms of organisms (e.g., anhydrobiotic nematodes) to become active and vulnerable to the reapplied treatment (HUHTA *et al.*, personal observation, BENGTSSON *et al.*, 1988). MORLEY *et al.*, (1983) have shown that repeated freezing stress (to −27 °C) results in only 40–60 % bacterial mortality in shortgrass prairie soil. As a biocide we elected to apply a combination of carbofuran and naphthalene, which had proven effective in a previous study (WRIGHT & COLEMAN, 1988).

Our objectives were to test and compare the effectiveness and side effects of these techniques, and at the same time to look for patterns of response to the different defaunation methods that might reflect faunal effects on soil processes. We duplicated our experiments with soils from 2

sites: a deciduous forest in Georgia, southeastern USA, (Exp. I) and a boreal coniferous forest in central Finland (Exp. II).

2. Materials and methods

2.1. Experiment I

A randomized complete block design was used, with 4 treatments, 8 blocks, and one replicate per cell. The treatments were: repeated microwaving (μWAVE), repeated freezing-thawing and drying (FTD), biocide applications (CHEM), and an untreated control (CON).

On May 5, 1987, intact cores of litter, humus, and mineral soil, 15.2 cm^2 in area and 6 cm deep were collected into sections of plastic pipe from a riparian deciduous forest in the Horseshoe Bend Research Area of the University of Georgia (Athens, Georgia, USA). The soil is a Typic Udipsamment, with 80% sand (53–2,000 μm), 18% silt (2–53 μm), and 2% clay (less than 2 μm). Cores were taken in blocks of 4, with the members of each block as close together as possible. Each core was wrapped in foil and transported to the laboratory.

The fresh mass of the cores was equalized to 64.6 g (average 42.4 g oven-dry) by removing mineral soil from the bottoms of heavier samples, and then a piece of 63 μm mesh nylon screen was glued to the bottom of each core. Within blocks, cores were randomly assigned to treatments, which were applied as described below. Day numbers are relative to May 11, 1987, which was designated day 0 for this experiment.

Microwaving was applied for 3 min at full power (380 W measured output) in an ordinary microwave oven designed for food preparation (2,450 MHz). Temperatures in the cores during microwaving rapidly rose to near 100°C in 1 min, and stayed around 100°C for the remaining 2 min. Microwaving was applied on day −5 and again on day −1; in the interval between microwavings the cores were watered once with deionized water, covered, and left at room temperature. After the second microwaving, cores were stored at 15°C.

FTD cores were frozen at −80°C on day −6, thawed on day −5, placed in a 60°C oven to dry on day −4, and re-frozen (after drying 32 h) on day −3. On day −2 these cores were wetted repeatedly and incubated in a saturated atmosphere at 30°C to try to bring them to their original moisture content. Wetting continued on day −1, when the cores were placed in a 15°C incubator.

CHEM and CON treatments were stored at 15°C between days −5 and 0. Carbofuran (2.8 $g \times m^{-2}$ active ingredient) and naphthalene (100 $g \times m^{-2}$), both in granular form, were added to the surface of the CHEM cores on day 0. Biocides were reapplied on day 16 at the same rates.

On day 0, all replicates were watered with deionized water to their original fresh mass; however, some of the water leached through the μWAVE and FTD cores. This procedure was repeated on day 1. From day 0 onwards all samples were incubated at 15°C in the dark, each treatment placed in a cardboard box having a ventilation opening with 63 μm mesh but otherwise taped closed.

Respiration was measured on days, 1, 3, 8, 15, 22, 29, 36 and 43 by placing the cores in sealed jars and trapping the CO_2 evolved for 24 h in 10 ml of 1 M NaOH (ANDERSON, 1982). On days 2, 16, 30, and 44, the cores were placed in funnels and watered with deionized water until 22 ml of leachate was obtained. The water holding capacity (amount of water absorbed during leaching) was noted, and *p*H, NH_4^+, NO_3^-, and total Kjeldahl N were determined from the leachates. Ammonium and nitrate in leachates, and N as NH_4^+ in microkjeldahl digests of 5 ml of leachate were determined colorimetrically on a Tecator flow-injection analyser (BREMNER & MULVANEY, 1982; KEENEY & NELSON, 1982; TECATOR, 1981, 1984 a, 1984 b). "Organic" N was calculated as KJELDAHL N minus NH_4^+-N.

Upon termination of the experiment (day 46), a subsample was taken from the center of each core using a 1.8 cm diameter cork borer. This subsample was mixed and subdivided as follows (fresh masses): 4 g was extracted with 20 ml of 2 M KCl and analysed colorimetrically for NH_4^+ and NO_3^- concentrations (TECATOR, 1984), and 5 g was extracted on Baermann funnels and examined for nematode populations (VAN GUNDY, 1982; WRIGHT, 1988). The soil used in the nematode extractions was then oven-dried (45°C) for 48 h and weighed to determine initial soil moisture content. After removal of the subsample, the rest of the core was placed in a high-gradient extractor for 7 d to extract microarthropods (MACFADYEN, 1961; MERCHANT & CROSSLEY, 1970). A non-quantitative survey for enchytraeids was made by dissecting several of the dried cores from the microarthropod extraction procedure in water.

2.2. Experiment II

Here we note only details in which the procedures in Exp. II differed from Exp. I.

Intact cores 25 cm^2 in area and approximately 6 cm deep were taken from a Norway spruce stand with a podsolic (spodosolic) raw humus soil in Jyväskylä, Central Finland, on July 21, 1987 (day −6); and were assigned to treatments in a completely randomized design. There were 6 replicates per treatment.

Soil masses were equalized to 33 g (fresh; average 16.8 g dry), after which only a few samples contained mineral soil in the bottoms, the rest containing only litter and humus. Microwaving was applied at full power (619 W measured output) for 3 min on day −6 and for 2 min on day −1. Temperatures in microwaved cores approached 100 °C in less than 30 s. Biocides, the amounts of which were corrected to be the same per unit area as in Exp. I, were applied on days 0 and 9. A third freezing cycle was added to the FTD treatment between rehydrations on days −2 and −1.

Respiration in Exp. II was measured with a URAS 7N carbon analyser. Samples were enclosed in glass jars for 1 h, and CO_2 content at the beginning and end of the incubation was determined by withdrawing 5 ml air samples through rubber septa in the lids of the jars. Cross-calibration indicated that the alkali absorption and carbon analyser results were comparable. On days 2, 15, and 29, the microcosms were watered to obtain 40 ml of leachate (chosen to be comparable to the volume in Exp. I on a per-unit-area basis). The same parameters were measured, except that NO_3^- in leachate was measured only on day 2, and, as is usual in this soil, was found to be extremely low.

At the termination of the experiment (day 44), two subsamples were taken from each core using a 1.5 cm diameter cork borer, one for the extraction of nematodes (wet funnels; SOHLENIUS, 1979), and another for the analysis of NH_4^+. The latter subsample was mixed with 70 ml of 2 M KCl for 15 min, filtered and analysed colorimetrically. In this experiment, total Kjeldahl N in the soil KCl extract was also analysed.

The CON cores were then divided into 2 sets for further faunal extractions: 3 cores were extracted for microarthropods (high gradient extractor; MACFADYEN, 1961) and the remaining 3 for enchytraeids (wet funnel technique; O'CONNOR, 1962). Cores from the other treatments were extracted for microarthropods only, as previous experiments had shown enchytraeids to be highly sensitive to all the defaunation methods.

We present our results for respiration and nutrients in leachate in $\mu g \times cm^{-2}$. To convert to $g \times m^{-2}$, divide by 100; to express nurients as concentrations in leachate in $\mu g \times ml^{-1}$ (ppm), multiply by 0.69 for Exp. I or by 0.625 for Exp. II.

3. Results

3.1. Experiment I: Deciduous Forest, Georgia

Animal populations: At the termination of the experiment the control samples retained an abundant and diverse soil fauna (table 1). Nematodes had been most effectively eliminated by microwaving, while there were considerable populations of nematodes in FTD and CHEM. All defaunated samples were contaminated by astigmatid mites (*Tyrophagus:* Acaridae), which proved to be abundant in the incubation room. FTD, μWAVE, and CHEM contained mostly or entirely mites that were likely postdefaunation contaminants. Control cores, on the other hand, contained contaminants in numbers roughly equal to those in FTD and μWAVE, but the difference in total microarthropods between CON and other treatments was made up of diverse Oribatida, Prostigmata, Mesostigmata, Collembola, and other arthropods from the original fauna. CON cores also contained an average of 12.7 ± 14.3 enchytraeids (1.0 per cm^2), while 1 enchytraeid was found in one of 4 CHEM cores examined. μWAVE and FTD were not sampled for enchytraeids.

Table 1. Soil faunal populations at the end of the experiments: mean (s.d.).

		Microwave	FTD	Biocide	Control
Nematodes	Exp. I	0.6^a (1.7)	102^b (189)	23^b (17)	228^c (141)
(per g dry soil)	Exp. II	0^a (0)	173^b (100)	91^b (73)	238^b (232)
Microarthropods	Exp. I[1]	6.8^a (3.6)	6.7^a (3.4)	3.6^a (1.3)	16.3^b (6.2)
(per cm^2)	Exp. II	0^a (0)	0^a (0)	0.2^b (0.2)	21.2^c (6.7)

[a,b,c] Within any single row, means followed by different letters are significantly different ($P < 0.05$, Tukey's HSD)
[1] Astigmatid and a few other contaminant mites. See text.

Respiration: Initially all defaunation techniques reduced the amount of CO_2 evolved, μWAVE virtually to zero, followed by FTD and CHEM. Subsequently, FTD experienced a brief flush, but otherwise respiration remained depressed in FTD and especially μWAVE throughout the experiment (fig. 1). CHEM experienced a flush of respiration in excess of the control after reapplication of the chemicals.

Leaching of nitrogen: Two of the CON cores showed widely divergent patterns of nitrogen

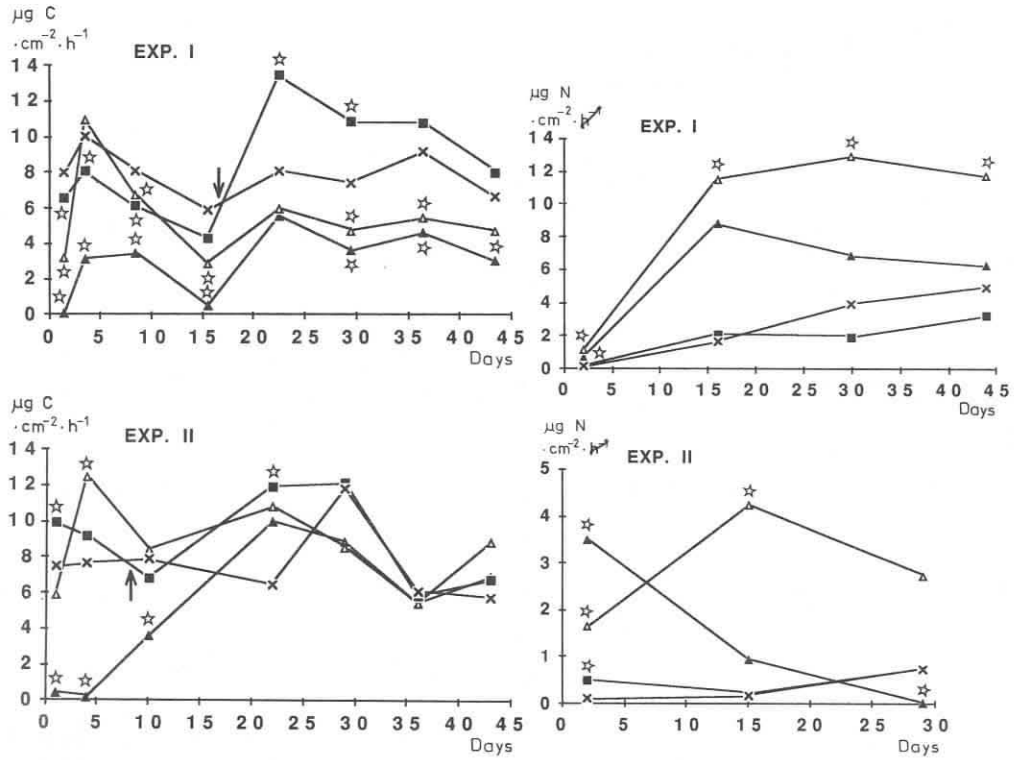

Fig. 1. CO_2-evolution in the two soil types (Exp. I: deciduous forest, Georgia, USA; Exp. II: coniferous forest, central Finland) Asterisks indicate significant differences from the control ($P < 0.05$); the arrow indicates the reapplication of chemicals to the biocide treatment. Treatment symbols: (X) UNTREATED CONTROL, (▲) MICROWAVE, (△) FTD, (■) BIOCIDES.

Fig. 2. Leaching of ammonium-N from the two soil types. Symbols as in fig. 1.

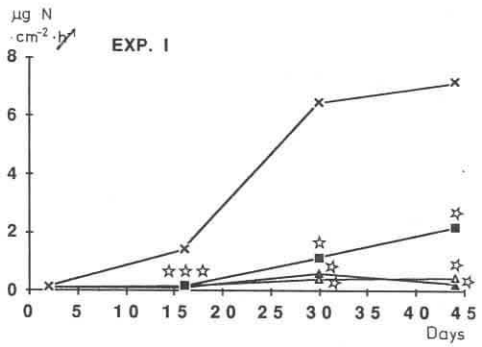

Fig. 3. Leaching of nitrate-N in Exp. I. Symbols as in fig. 1.

leaching when compared with the remaining six. Losses of NH_4^+-N and organic N were roughly 5 times greater, and these differences persisted over time. The same two cores also had aberrant leachate *p*H values, averaging about 0.8 *p*H units higher. We tentatively attributed these differences to the presence of earthworms in these 2 samples, since earthworms are known to affect these parameters (HUHTA *et al.*, 1986; SCHEU, 1987). In any case, these 2 cores were regarded as outliers and were excluded from the analyses of NH_4^+, organic N, and *p*H. They did not differ from other

CON cores in their respiration, NO_3^- leaching, or water holding capacity, and they are included in these analyses.

Mineral forms of N in leachates were initially low in all treatments. CON cores showed a trend of gradual increase in NH_4^+-N losses (fig. 2), and a strong increase in NO_3^--N (reaching the level of NH_4^+-N by the end of the experiment; fig. 3). FTD had increased amounts of NH_4^+-N in leachate throughout the experiment. μWAVE initially increased NH_4^+ in leachate, especially on day 16, but thereafter losses declined and approached CON values. In CHEM the amounts were similar to the CON (fig. 2). NO_3^- remained very low in FTD and μWAVE throughout the experiment, while CHEM resulted in increased values towards the end (fig. 3).

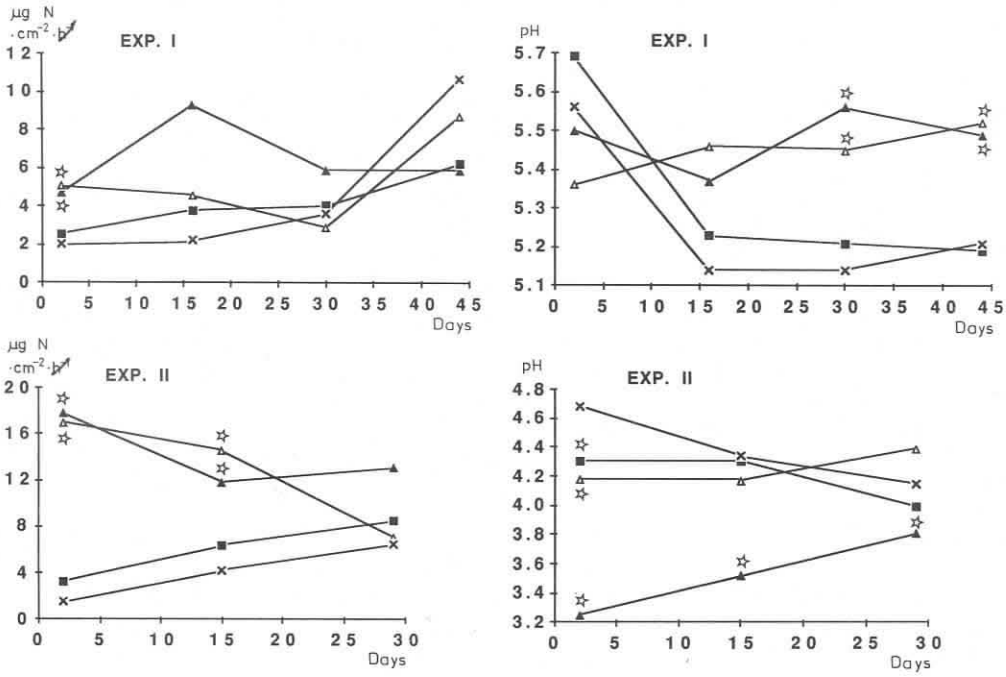

Fig. 4. Leaching of organic N (calculated as Kjeldahl-N minus ammonium-N) from the 2 soils. Symbols as in fig. 1.

Fig. 5. The *p*H of leachate from the two soils. Symbols as in fig. 1.

Few differences in organic N were detectable between treatments. Excluding the two CON units having exceptionally high losses, μWAVE and FTD had significantly higher values than CON on day 2 (fig. 4).

***p*H of leachate:** Initially there was little difference between treatments, but by day 16 the *p*H of CHEM and CON treatments had decreased, and remained consistently below μWAVE and FTD for the rest of the experiment (fig. 5).

Water holding capacity: The FTD and, especially, μWAVE cores consistently absorbed less water than did the CON cores ($P < 0.001$, data not shown). On average, μWAVE absorbed 6.1 ml, FTD 7.0 ml, and CON 9.5 ml during the leaching procedures. CHEM (average 9.1 ml) did not differ from CON.

Soil properties at end of experiment: The amounts of mineral N in soil paralleled those measured in the leachates (table 2). Water content was lowest in μWAVE soil, with less than half of that in CON. FTD had intermediate moisture, while CHEM did not differ significantly from CON.

Table 2. Soil properties at the end of the experiments: mean (s.d.)

		Microwave	FTD	Biocide	Control
NH_4^+-N (μg/g)	Exp. I	84^a (30)	129^b (57)	42^a (28)	64^a (36)
	Exp. II	11^a (13)	35^b (37)	55^b (34)	38^b (27)
NO_3^--N (μg/g)	Exp. I only	0.44^a (.99)	0.06^a (.11)	0.37^a (.74)	2.53^b (2.30)
Organic N[1] (μg/g)	Exp. II only	94^a (22)	95^a (18)	180^b (50)	200^b (59)
Soil moisture	Exp. I	29.9^a (6.9)	44.4^b (5.0)	61.3^c (8.4)	71.3^c (17.3)
(% of dry mass)	Exp. II	75^a (8.7)	202^b (25)	233^b (15)	234^b (15)

[a,b,c] Within any single row, means followed by different letters are significantly different ($P<0.05$, Tukey's HSD)

[1] Total Kjeldahl N minus NH_4^+-N in KCl extract

3.2. Experiment II: Coniferous Forest, Finland

The results of the second experiment, with podsolic (spodosolic) coniferous forest soil, were in broad outline similar to Exp. I.

No nematodes were found in μWAVE, while in FTD their numbers were nearly as high as in CON (table 1). Substantial numbers were also found in CHEM. A few microarthropods (oribatid mites) were found in CHEM; none in μWAVE or FTD. There was no contamination by Astigmata. In CON there was a diverse fauna of microarthropods (table 1), enchytraeids (81.3 ± 29.0 per extract, or 3.8 ind. per cm^2) and earthworms (5 emerged in the 3 microarthropod extractions, normally an inefficient method for extracting earthworms).

Microwaving reduced respiration to nearly zero for the first four days, but after day 15 there was no obvious difference from CON (fig. 1). As in Exp. I there was a transitory flush on day 4 in FTD, and after the second application of biocides in CHEM. CHEM also showed slightly elevated respiration on day 1.

Levels of NH_4^+ leaching were much lower than in Exp. I, but the trend in CON was similar (fig. 2). FTD increased NH_4^+ leaching even more dramatically than in Exp. I, including the first samples on day 2. In μWAVE there was also a very large increase initially, but this effect quickly declined, and by day 28 NH_4^+ losses from this treatment were below CON. CHEM showed elevated NH_4^+ leaching on day 2, but afterward was hardly distinguishable from CON.

The trends in organic N in leachate were rather similar to those in NH_4^+: FTD and μWAVE had elevated amounts at first, but approached CON values by day 30 (fig. 4).

The *p*H of CON leachate in Exp. II was initially much lower than in Exp. I (fig. 5). All defaunation treatments, especially μWAVE, showed even lower *p*H values on day 2. Subsequently CON displaying a decreased trend in leachate *p*H and treatments FTD and CHEM did not differ from control. The *p*H of μWAVE remained low.

μWAVE had lower capacity to absorb water, as in Exp. I ($p<0.05$).

At the termination there was less KCl-extractable NH_4^+-N and organic N in μWAVE soil than in other treatments (table 2). FTD also exhibited reduced concentrations of organic N in the KCl extract. μWAVE had lower soil moisture content than CON.

4. Discussion

All of the defaunation techniques we examined had distinctive, measurable and potentially important side-effects on soil properties. These side-effects sometimes varied with soil type, and also vary with soil pretreatment such as mixing (see WRIGHT *et al.*, 1989). Distinguishing between side-effects of defaunation and direct effects of fauna is difficult unless side-effects can be controlled. Since the development of a perfect defaunation technique seems unlikely,

we recommend that when faunal effects are to be investigated via a defaunation experiment, an additional control treatment which has been defaunated and then refaunated should be included in the experiment. Microbial reinoculations and a recovery period should also be considered.

Our experiments showed that microwaving is an effective defaunation technique, which initially depressed virtually all biological activity. On the other hand, nematodes were occasionally recovered from microwaved treatments, and in the experiments by HUHTA *et al.*, (1988) and SETÄLÄ *et al.*, (1988), FTD resulted in complete defaunation. Thus no one of the methods tested proved to be completely consistent or reliable. Nematodes are among the most difficult metazoans to eliminate, because they can survive harsh conditions in an anhydrobiotic state (FRECKMAN, 1978; DE MEURE *et al.*, 1979). Some of the protozoan fauna survived all treatments; these were not quantitatively enumerated in our studies. However, ciliate protozoans were observed to be especially abundant in samples where nematodes were few or absent, suggesting an interaction between these groups in soil.

If an extended experiment is planned (considering the short generation time of some nematodes), even a slight chance of survival or contamination may result in large populations of nematodes developing during the experiment. In general, small amounts of soil are easier to defaunate completely than large amounts. If long-term total defaunation is required, excess replicates should be prepared and monitored so that contaminated replicates can be removed. Under some circumstances, contamination by nematodes can be checked by inspecting the leaching waters under a dissecting microscope. Care should also be taken to avoid contamination by astigmatid mites and other colonizing fauna.

Microwaving resulted in unidentified physical changes in the soil, most notably reflected in reduced water holding capacity. Casual examination by dissecting or scanning electron microscope revealed no obvious micromorphological effects. Organic matter and clays normally show a hysteresis response to wetting and drying – they are more resistant to wetting once they have been dried, and vice versa (ELLIOTT *et al.*, 1986). This cannot fully explain the reduced wettability of μWAVE soils, however, since FTD showed greater wettability despite being more severely dried. Differences between μWAVE and other treatments should be interpreted with care because different moistures of the samples may have caused the differences observed, e.g., in respiration.

FTD also caused physical and chemical disturbances in the soil. There was a more rapid recovery in the CO_2 production, including a brief flush during the first week, indicating some perturbation and recovery of microbial activity. NH_4^+ leaching was greatly elevated. The wettability of FTD treatments was lower than in the CON. FTD was as effective as microwaving in eliminating microarthropods and enchytraeids, but in most cases, including the recent experiments by HUHTA *et al.*, (unpubl.), elimination of nematodes by FTD was less effective.

The chemical reduction of soil fauna with carbofuran and naphthalene had the fewest side-effects on soil physico-chemical parameters, but its effectiveness in the elimination of fauna was rather low and selective. The transitory flush of CO_2 evolution observed in experiments I and II after reapplication may have been due to microbial degradation of the biocides (WILLIAMS & WIEGERT 1971; FELSOT *et al.*, 1981; CAMPER *et al.*, 1987; NEWELL *et al.*, 1987; BLAIR *et al.*, in press). The extra production of CO_2-C corresponds to less than 10% of the C in the chemicals. The need to reapply biocides that produce incomplete defaunation, with associated effects on the composition and activity of the soil microbial community, perturbation of microbially mediated nutrient transformations, and the difficulty of a "refaunation" treatment are all serious disadvantages of this technique. Other methods may be preferable to the use of chemicals in controlled laboratory studies, however, biocides may be appropriate in some field experiments where other methods are not easily applied (FINLAY, 1985; SEASTEDT & CROSSLEY, 1983).

While responses to the defaunation treatments were in many ways similar in the soils from the 2 forest sites, some differences are worth noting. Most of these differences are probably attributable to the predominance of organic matter in the Jyväskylä cores (Exp. II) versus the presence of a mineral layer in the Athens cores (Exp. I), or to differences in the initial values of soil parameters. For example, in the Jyväskylä system, inorganic N is strongly ammonium-dominated, with little nitrate leaching. This pattern has been confirmed by periodic sampling in a variety of experiments (HUHTA *et al.*, 1988 and unpublished data; WRIGHT *et al.*, 1989). In the Athens soil, however,

nitrate formation can be considerable (fig. 3, COLEMAN & WRIGHT, personal observation). Reduced nitrate leaching in all three defaunation treatments in Exp. I suggests either an interaction between soil fauna and nitrifying bacteria, or that nitrifying bacteria are sensitive to all of the defaunation methods. Most of the total N in leachate was in organic form in Jyväskylä soil, and organic N loss was more strongly increased by microwaving and FTD in Exp. II than in Exp. I.

One of the most striking differences was seen in the response of *p*H to the defaunation treatments. In Exp. I, μWAVE and FTD resulted in elevated *p*H values, but in Exp. II exactly the reverse was true, most notably in μWAVE (fig. 5). *p*H is affected by complex interactions of several variables and processes in the soil, whereby contents of NH_4^+ and NO_3^-, cation exchange capacity, and production of organic acids, for example, all play roles (BINKLEY & RICHTER, 1987). The *p*H should therefore not be considered separately but against this background.

More evidence about the role of fauna in soil processes is available in the accompanying paper (WRIGHT *et al.*, 1989) and in the studies of HANLON & ANDERSON (1979), ANDERSON & INESON (1983), ANDERSON *et al.*, (1983a), BÅÅTH *et al.*, (1981), BENGTSSON *et al.*, (1988), COLEMAN & HENDRIX (1988), HÅGVAR (1988), SETÄLÄ *et al.*, (1988), and HUHTA *et al.*, (1988). The role of macrofauna, especially earthworms, remained tentative in our experiments (but see HUHTA *et al.*, 1986; SCHEU 1987), nevertheless, the suggestion that earthworms and perhaps other macrofauna can have an overwhelming effect on soil processes is intriguing. More experiments to compare the impacts of meso- and macro-fauna are in progress and will be published in the near future (HUHTA *et al.*, unpubl.).

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Synopsis: *Original scientific paper*

Huhta, V., D. H. Wright, & D. C. Coleman, 1989. Characteristics of defaunated soil. I. A comparison of three techniques applied to two different forest soils. Pedobiologia **33**, 415–424.

We tested the effects of three convenient defaunation methods; microwaving, deep-freezing + drying, and biocide application (carbofuran + naphthalene); on CO_2 evolution, nitrogen leaching, and other parameters. Experiments were carried out on intact cores from two different forest soils, a deciduous forest in Georgia, USA, and a coniferous forest in Finland. Microwaving was most effective in eliminating the fauna, followed by freezing. On the other hand, microwaving also had strong side effects on soil properties such as lowering water holding capacity. Soil respiration was initially reduced nearly to zero by microwaving and freezing. Flushes in respiration were observed after a few days in the freezing treatment and after reapplication of chemicals in the biocide treatment. Leaching of NH_4^+ was strongly increased in the microwaving and freezing treatments. The trends were mainly similar in the two soils, but differences were also detected, e.g. in *p*H and leaching of NO_3^-. It was concluded that when faunal effects are to be investigated using a defaunation experiment, control treatment should be included that has been defaunated and then refaunated.

Key words: Forest soil, soil fauna, defaunation, microcosms, techniques.

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